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Artículo original

Nosographic performance of the red cell distribution width (RDW) for the diagnosis of thalassemia

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RESUMEN

Antecedentes: el diagnóstico definitivo de talasemia se fundamenta en pruebas de laboratorio relativamente complejas y, por tanto, en la práctica clínica rutinaria estos síndromes pueden subestimarse.
Métodos: se incluyeron 500 individuos identificados en forma consecutiva en los Laboratorios Clínicos de Puebla por presentar hipocromía (HCM<24 pg) o microcitosis (VCM < 75fL en mujeres y <80fL en hombres), con o sin anemia, a lo largo de 16 meses. En todos ellos se investigó deficiencia de hierro y talasemia α y β por métodos definitivos.
Resultados: del total de los 500 pacientes incluidos en el estudio, 394 (78.8%) mostraban deficiencia de hierro, en 37 se documentó talasemia β y en 11 talasemia α; en los restantes 58 casos (11.6%) no pudo establecerse un diagnóstico definitivo. El ancho de la distribución eritrocitaria (RDW) fue significativamente menor en pacientes con talasemia que en quienes tuvieron deficiencia en hierro y, por sí solo, este parámetro mostró alta especificidad y sensibilidad nosográficas para el diagnóstico de talasemia α o β.
Conclusiones*:* los síndromes talasémicos deben sospecharse en individuos con microcitosis o hipocromía, con o sin anemia, con valores muy bajos del RDW. En estos individuos deben indicarse pruebas confirmatorias.
Palabras clave: talasemia, anemia, hemoglobina, RDW

ABSTRACT

Background: The definite diagnosis of thalassemia is based upon relatively complex laboratory tests, hence, these syndromes might be underestimated in the routine clinical setting.
Methods: 500 consecutive individuals identified in Laboratorios Clínicos de Puebla with red blood cells showing either hypochromia (MCH<24 pg) and/or microcytosis (MCV <75 fl in women or <80 fl in man), with or without anemia, were prospectively accrued in this study, along a 16 month-period. Iron deficiency, β and α-thalassemia were searched by definite methods.
Results: Out of the 500 consecutive cases with red blood cell hypochromia or microcytosis, 394 (78.8%) were found to have iron deficiency, 37 cases had β-thalassemia, 11 cases had a-thalassemia, while in 58 cases (11.6%) a definite diagnosis could not be established. Red cell distribution width (RDW) was significantly lower in the thalassemic patients than in the iron deficient group, and it proved to bear high nosographic sensitivity and specificity for the diagnosis of either α or β thalassemia.
Conclusions: The thalassemic syndromes should be suspected in individuals with red blood cell microcytosis and / or hypochromia, with or without anemia, showing very low RDW values. These individuals should be further tested for thalassemia.
Key words: Thalassemia, anemia, hemoglobin, RDW

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The thalassemias result from impaired synthesis of one or more of the polypeptide chains of the normal human hemoglobins; this primary feature is a quantitative one and contrasts with the qualitative changes of hemoglobin structure that characterize the hemoglobinopathies.[1] Thalassemia is considered the most common genetic disorder worlwide: As far as beta (β-thalassemia is concerned, about 3% of the world´s population (180 million people) carry β-thalassemia genes,[2] these genes being

particularly prevalent in inhabitants of Italy and Greece, the highest prevalence of the carrier state being found in Sardinia (34%), the delta region of the Po river near Ferrara (20%) and Sicily (10%). The prevalence of the carrier state of β-thalassemia in other parts of the world does not seem to be low and there are data which suggest that the condition is not infrequent;[3,4] in addition, clusters of the condition in Mexico with up to 15% prevalence have been identified,[5,6] with data which suggest that β-thalassemia genes in some places are autochthonous[5] and in others imported from the Mediterranean area.[6] Concerning α thalasssemia in México, the information is even more scant: α thalasssemia has been found to be responsible for 1% of the hypochromic microcytic anemia in México, this figure being about one half of that of β-thalassemia.[4] The thalassemic syndromes may result in red blood cell hypochromia and / or microcytosis with or without anemia, conditions that can mimic iron-deficiency states.[1] In geographic areas where both conditions are common, routine markers useful in their differential diagnosis are necessary. We report herein on the nosographic performance of the red cell distribution width (RDW) as a single presumptive marker for the diagnosis of thalassemia.

MATERIAL AND METHODS

Blood samples from 500 consecutive individuals identified in Laboratorios Clínicos de Puebla, with red blood cells showing either hypochromia (MCH<24 pg) or microcytosis (MCV <75 fl in women or <80 fl in man), with or without anemia, were prospectively accrued in the study along a 16 month-period. Written informed consent was obtained from all individuals.

Laboratory tests

a) Routine red blood cell analysis

Values for hemoglobin (Hb), hematocrit, red cell counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red cell distribution width (RDW) were measured in all subjects in an automated hematology analyzer (Hmx® Beckman Coulter).

b) Assessment of the zinc-protoporphyrin (ZPP) complex

PPZ levels were determined in all samples with the ProtoFluor ® Reagent Kit (Helena Laboratories) following the instructions of the manufacturer. Results greater than the cut-off level of 80 mmol ZPP/mol heme were used to define iron deficiency.

c) Quantification of Hemoglobin A_2 (HbA_2)

In all samples in which iron deficiency was excluded (PPZ levels below 80 mmol/mol heme), the quantification of HbA_2 was performed by ionic exchange column chromatography using the commercial β-Thal HbA_2 Quick Column Kit® (Helena Laboratories) according to provided instructions. Levels of HbA_2 above 3.8% were considered as indicative of β-thalassemia.

d) DNA extraction

High molecular weight DNA was extracted from the samples remaining after exclusion of β-thalassemia according to standard protocols.[7]

e) Detection of a gene mutations and deletions

The genotype $-a^{4.2}$ was determined with primers described in 8. The corresponding PCR conditions are outlined in (9). The genotype $-a^{3.7}$ was analyzed (10) and homozygous cases were confirmed with the help of a multiplex PCR (11). Additional deletional a-Thal (a^{SEA}, a^{THAI}, $a^{20.5}$, a^{MED}, a^{FIL}) included in this multiplex PCR were also screened for.[11] Non-deletional a-Thal mutations ($a2^{Hph}$, $a2^{Nco}$, a^{TSaudi}, $a\_^{Nco}$) were searched by selective amplification[12] and $a2^{Hph}$ mutations were confirmed by direct sequencing.[13]

f) Statistical analysis

Mean values of the different parameters in the three groups were compared with the use of the *t* test for non-paired observations. Sensitivity, specificity, relative risk and odds ratio of several parameters for the identification of patients with thalassemia were estimated in contingency tables. Recievers Operating Characteristics (ROC) curves were estimated for MCV, MHC and RDW with the aid of the MedCal® software. Significant differences were considered when a was less than 0.05.

RESULTS

Patients' distribution

Out of the 500 consecutive cases with red blood cell either hypochromia or microcytosis, 394 (78.8%) were found to have iron deficiency, 48 cases (9.6) had thalassemia (37 cases of β-thalassemia and 11 cases of α thalassemia),

whereas in 58 cases (11.6%) a definite diagnosis could not be established.

Red cell features

The values of hemoglobin MCV, MHC, RDW, PPZ and HBA_2 obtained in the three groups of patients are summarized in table 1. Most striking was the finding that the values of the RDW, but not those of the MCV and MHC, were significantly different in the iron deficiency group than in the thalassemia patients. As expected, hemoglobin values were significantly lower in the iron deficiency group, both in the female and the male patients, than in the α or β thalassemia subjects. Inasmuch as the elevation of the PPZ complex was used as one of the diagnostic criteria for iron deficiency, its mean value was, as expected, much higher in this group.

Table 2 shows the sensitivity and specificity of the RDW values to discriminate patients with thalassemia, at three different cutoff levels, and figure 1 depicts the corresponding ROC curve. The individual association of low RDW values with thalassemia, either α or β, is reflected in the χ^2 values and the figures of the relative risk and odds ratio at all three cutoff values. None of the other red cell parameters that were analyzed (Hb, MCV or MCH), even approximated acceptable values of sensitivity and specificity (data not shown).

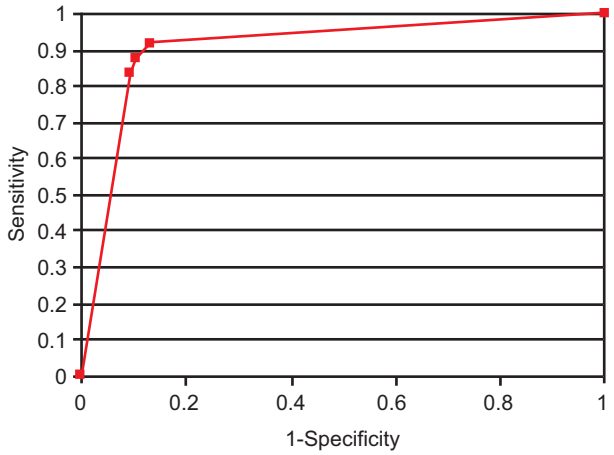

Figure 1. ROC Curve displaying the nosographic performance of the RDW for the diagnosis of thalassemia at three different cutoff values (see Table 2)

Table 1. Salient features of the red blood cells in the individuals identified with iron deficiency, β-thalassemia and α-thalassemia

Parameter	*Iron Deficiency*	*p**	*β-Thalassemia*	*p***	*α-Thalassemia*
Hemoglobin, g/dL	9.56 ± 1.98§	9.36E-16	12.04 ± 1.45	ns	12.62 ± 2.05
MCV, fL	66.82 ± 5.67	ns	66.22 ± 4.09	0.00225	71.48 ± 4.72
MHC, %	20.71 ± 2.49	ns	20.68 ± 1.43	0.00646	22.69 ± 2.17
RDW,%	19.07 ± 3.48	1.53E-32	15.11 ± 1.09	ns	14.34 ± 2.03
ZPP, mM	156.82 ± 52.55	3.48E-69	58.92 ± 16.72	4.27E-15	42.45 ± 12.01
HBA_2, %			4.97 ± 0.81	7.75E-03	2.66 ± 0.40

§Results are expressed as mean ± standard deviation.
p values obtained by the *t* test
p* Iron deficiency versus all thalassemias
p** b-thalassemia versus a-thalassemia
ns = non significant

Table 2. Nosographic performance of RDW for the diagnosis of Thalassemia

Cutoff Value	*Sensitivity, %*	*Specificity, %*	$\chi^2_{(Yates)}$	*p* (χ^2)	*Relative Risk*	*Odds Ratio*
RDW ≤ 15.7	83.3	90.52	157.81	<1.0 e-8	23.78	47.76
RDW ≤ 16.0	87.5	89.7	164.8	<1.0 e-8	30.87	61.46
RDW ≤ 16.2	91.6	86.8	151.18	<1.0 e-8	39.92	72.73

DISCUSSION

It is known that the most frequent cause of anemia as the primary complain in México is iron deficiency, which represents 69.6% of patients;[4,14] moreover, iron deficiency anemia represents 6% of all patients studied and treated at our institution.[14,15] In the present study, perhaps because individuals with or without anemia were accrued, iron deficiency anemia represented up to 78.8% of all cases of microcytosis and/or hypochromia. We also found that the thalassemic syndromes accounted for almost 10% of such cases and that β-thalassemia was at least three times more frequent than α-thalassemia in this sample. It is important to mention that types of α-thalassemia other than the ones that we have searched for in this paper may account for some additional cases of thalassemia, that were included in the subset of individuals in which a definite diagnosis could not be established; hence, it is possible that we may be underestimating the prevalence of α-thalassemia in this study. Nevertheless, it is evident that either α or β thalassemia is not an infrequent condition in our population and therefore, a reliable surrogate marker for its detection might prove useful in the routine clinical setting.

The analysis of the nosographic performance of the RDW values resulted in high levels of sensitivity and specificity for the correct identification of patients with either α or β-thalassemia. The relative risk values for the different cutoff points of the RDW should be interpreted as the number of times that the probability of thalassemia in an individual fulfilling the criterion (RDW ≤ cutoff) increases in comparison to an individual not fulfilling it; while the value of the odds ratio is a measurement of the information gain in terms of times. Taken altogether, these figures suggest that the value of the RDW is a reliable marker of thalassemia in individuals with microcytosis or hypochromia. As with most diagnostic procedures, if a stringent cutoff value is used, the finding becomes highly specific but not as sensitive and, conversely, if the decision is based in a higher cutoff level, the test gains in sensitivity but sacrifices specificity.

In the routine clinical setting, the RDW might be used at the 16.2% cutoff level as a screening procedure for thalassemia. This should identify correctly more than 9 out of 10 patients actually having thalassemia, while it will become an indication for unnecessary further testing in less than 15% of subjects not having thalassemia. This predictive value might prove very useful in situations where confirmatory tests for thalassemia are not readily available, such as in many communities of developing countries, but also might be suitable to rule out thalassemia in other scenery. In a study informed from the United States, there are data showing that the most frequent cause of anemia in the general practice, the "common anemia" is thalassemia, since β- added to α-thalassemias are more frequent than iron deficiency anemia;[16] hence, a simple routine test with a reliable predictive value could prove useful for decision making.

REFERENCES

1. Ruiz-Reyes G. Hemoglobinopatías y talasemias. In: Ruiz-Argüelles GJ (editor). Fundamentos de Hematología. 3a ed. México: Médica Panamericana, 2003;p:132-154.
2. Lukens JN. The thalassemias and related disorders: Quantitative disorders of hemoglobin synthesis. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM (eds). Wintrobe´s Clinical Hematology. 10th ed. Baltimore: Williams & Wilkins, 1999;p:1405-1448.
3. Ruiz-Reyes G.: Abnormal hemoglobins and thalassemia in México. Rev Invest Clín Méx 1998; 50:163-170.
4. Ruiz-Argüelles GJ, López-Martínez B, Ruiz-Reyes G. Heterozygous beta-thalassemia: Not infrequent in México. Arch Med Res 2001;32:293-295.
5. Reyes-Cruz G, Ruiz-Reyes G, Hernández-Acasiete M. Identificación de un foco de talasemia beta en Tamiahua, Veracruz. Rev Invest Clín Méx 1990;42:189-192.
6. Lisker R, Ruiz-Reyes G, López G, Peral-López AM, Zárate G. Características hereditarias de la población mexicana. Estudio de una comunidad de origen italiano. Rev Invest Clín Méx 1966; 18:11-21.
7. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, 1989.
8. Baysal E, Huisman THJ. Detection of common deletional alpha-thalassemia-2 determinants by PCR. Am J Hematol 1994;46:208-213.
9. Reyes-Núñez V, Garcés-Eisele J, Jorge S, Kimura E, et al. Molecular Characterization of Alpha-thalassemia in the Mexican Population. Rev Invest Clin (in press).
10. Bergstrome-Jones AK, Poon A. Evaluation of a single-tube multiplex polymerase chain reaction screen for detection of common alpha-thalassemia genotypes in a clinical laboratory. Am J Clin Pathol 2002;118:18-24.
11. Tan ASC, Quah C, Low PS, Chong SS. A Rapid and Reliable 7-Deletion Multiplex Polymerase Chain Reaction Assay for a-Thalassemia. Blood 2001;98:250-251.
12. Kattamis AC, Camaschella C, Sivera P, Surrey S, Fortina P. Human alpha-thalassemia syndromes: detection of molecular defects. Am J Hematol 1996;53:81-91.
13. Dode C, Rochette J, Krishnamoorthy R. Locus assignment of human alpha globin mutations by selective amplification and direct sequencing. Br J Haematol 1990;76:275-281.

14. Ruiz-Argüelles GJ, Ramírez-Cisneros F, Rivadeneyra L, Ruiz-Delgado MA, Molina-Alavez A. La frecuencia de las anemias megaloblásticas en la práctica privada en Puebla, México: Experiencia de 17 años. Medicina Univ 1999;1:165-167.
15. Ruiz-Reyes G. Los síndromes talasémicos no son infrecuentes en la población mexicana y se subdiagnostican y confunden con deficiencias de hierro. Medicina Univ 1999;1:67-73.
16. Beutler E. The common anemias. J Am Med Assoc 1988; 259:2433-2437.